

TABLE 1

5	F344 Rat ♂ 4~8 weeks old
	↓
	Isolation of Hepatic Cells (Collagenase Perfusion)
10	↓
	Percoll Centrifugation
	↓
15	Hepatocytes
	6×10^5 cells / 3.5cm dish (without collagen coat)
	37 °C, 5% CO ₂ , 95% Air
	DMEM, 44mM NaHCO ₃ , 20mM HEPES, 0.5mg/l Insulin
20	10 ⁻⁷ M Dexamethasone, 30mg/l L-prolin
	penicillin and streptomycin
	↓ 2~3 hours
	Medium Change
25	DMEM, 10% FBS, 44mM NaHCO ₃ , 20mM HEPES
	0.5mg/l Insulin, 10 ⁻⁷ M Dexamethasone
	10mM Nicotinamide, 10ng/ml EGF
30	0.2mM L-ascorbic acid phosphate
	penicillin and streptomycin
	↓ 4 days
	Pre-confluent
35	Subculture with 0.02% EDTA
	1% DMSO

40 The same operations were carried out through systems added with additive factors such as FCS, nicotinamide, EGF, L-ascorbic acid-phosphate except for one, to investigate the effects of the individual additives on the hepatocytes and the non-parenchymal cells.

45 As a result of the above, a treatment with 0.02% EDTA caused the hepatocytes to detach them in the form of clusters, and as shown in Fig. 1 (first subculture $\times 29.4$), the clusters adhered to the dish within one or more days of subculture (Fig. 1(a): the second day of subculture), grew from the third day or so of subculture (Fig. 1 (b): the fifth day of subculture), and part of cells died and peeled off on seventh day or so of subculture (Fig. 1 (c): the eighth day of subculture).

50 About the eighth day or so of subculture and thereafter, the surviving hepatocytes grew (Fig. 1 (d): the 44th day of subculture), and in the case of most proliferative clusters, the number of hepatocytes increased 5-fold on the 41th day of subculture (Fig. 2).

55 Growth of the hepatocytes after subculture was confirmed from the increase in number of the hepatocyte clusters, incorporation of BrdU, and mitotic figure. Incorporation of BrdU was observed in many hepatocytes on the 30th day of subculture (Fig. 3 (a)). Figs. 3 (a), (b) and (c) show the 30th, the 45th and the 50th days of the first generation of subculture, respectively.

In Fig. 3(a), double staining ($\times 100$) was applied with BrdU (brown)-transferrin (red); Fig. 3(b) is based on stain ($\times 606$) with α_1 -antitrypsin (brown); and in Fig. 3(c), staining ($\times 606$) was applied with albumin (brown). In the hepatocytes continuing to grow after subculture, expression of albumin, α_1 -antitrypsin, and transferrin